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Conception rates at field level using osmanabadi buck semen cryopreserved with low density lipoproteins

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Abstract
Low density lipoprotein (LDL) was used as an alternative to whole egg yolk for cryopreservation of Osmanabadi buck semen and its effect on the conception rates at field level was studied. The semen collected from five Osmanabadi bucks was pooled and divided into four groups. Group I was extended with semen extenders containing egg yolk (10%). Group II, III and IV were extended with semen extenders containing 6%, 8% and 10% LDL, respectively and cryopreserved. The cryopreserved semen of each group was used for artificial insemination in goats in rural areas around Bengaluru (n=15). The conception rates recorded in group I, II, III and IV were 40%, 40%, 53.3% and 33.33% respectively. Statistically there was no significant (P = 0.7394) difference for conception rates between the groups studied.

Keywords: Low density lipoproteins, egg yolk, semen cryopreservation, osmanabadi buck, artificial insemination, conception rates

Introduction
The semen cryopreservation and artificial insemination have enabled the worldwide distribution of desired genetic lines at a reasonable cost. Semen cryopreservation extends the availability of sperm for fertilization, however, the fertilizing ability of the frozen–thawed sperm is reduced because of alterations in the structure and physiology of the spermatozoa (Salamon and Maxwell, 2000; Barbas and Mascarenhas., 2009) [14, 5]. These alterations can be prevented by controlling the rate of cooling and by adding cryoprotective agents to the semen extenders (Anand *et al*., 2015) [4].
Egg yolk is a common cryoprotective agent used in semen extenders, it exerts many adverse effects on sperm quality due to the presence of high-density lipoproteins and other factors that reduce the respiration and motility of spermatozoa. But low-density lipoproteins (LDL) is reported to be the key ingredient responsible for cryoprotective action of egg yolk. Thus, addition of only LDL to the semen extender rather than adding whole egg yolk, prevents the detrimental effects of other substances present in the egg yolk that deteriorates sperm quality (Anand *et al*., 2015) [4]. In this context, comparative effect of egg yolk and different concentrations of LDL on cryopreserved semen of Osmanabadi bucks used for artificial insemination in local breeds of goats was analysed.

Materials and Methods
Five Osmanabadi bucks of age 2-5 yrs and 3-4 BCS housed at Small Ruminants Semen Station, Veterinary College, Bengaluru were selected for the study. Fresh chicken eggs collected from Department of Poultry Science, Veterinary College, Bengaluru were used for extraction of LDL. The LDL was extracted from egg yolk as per the method described by Moussa *et al*. (2002) [11] and was used as an alternative to whole egg yolk in preparation of semen extender for semen cryopreservation of Osmanabadi bucks.
The semen extender for control group was prepared using Tris, citric acid, fructose, 4% glycerol and 10% egg yolk, where as in the semen extender for treatment groups, egg yolk was replaced by LDL at the concentrations of 6 percent, 8 percent and 10 percent. Semen was collected from all the five bucks by artificial vagina method. Semen ejaculates of all the bucks were pooled to eliminate the individual buck effect. Pooled semen was divided into four equal fractions. One fraction was diluted with extender for control group (Group I with 10% egg yolk) and other three fractions were diluted with extenders for three treatment groups (Group

II, III and IV with 6%, 8% and 10% LDL respectively) to obtain a sperm concentration of 100 X 10^6 sperm/ dose. The diluted semen of all the four experimental groups (group I, II, III and IV) were cryopreserved using liquid nitrogen.

The effect of egg yolk and different concentrations of LDL on cryopreserved semen was studied at field level by using the semen of four different groups for artificial insemination in the non-descript does in the rural areas in and around Bengaluru. A total of 60 non pregnant multiparous does were selected for artificial insemination (AI) programme. These animals were synchronised by short term estrus synchronization protocol using Progesterone impregnated sponges, synthetic $PGF_2\alpha$ (Cloprostenol) and GnRH (Buserelin acetate).

The synchronized does were segregated and 24 hours after sponge removal till AI, the owner was advised to observe for the signs of estrus like swishing of tail, frequent urination, squatting to urinate. Fixed time insemination was performed at 48-55 hours following Cloprostenol sodium (ESTRUMATE®) administration. The does were divided into four groups with 15 animals (n = 15) in each group. Each group was inseminated with semen cryopreserved with four different groups of extenders (group I, II, III and IV).

Cryopreserved semen with post thaw motility of 30% and above was used for AI and thawed semen was deposited intra cervically. Inj. Buserelin acetate (RECEPTAL® VET, MSD Animal health, Pune, India) @ 4 µg was administered intramuscularly at the time of insemination. The does were kept separately from bucks for 7 days after insemination. Pregnancy diagnosis using trans-abdominal ultrasonography (EASI-SCAN™, BCF Technology Limited, UK) using linear probe 5/7.5 Mhz was done after 30 -35 days of insemination. The conception rates were recorded in group I, II, III and IV. The conception rates obtained for four experimental groups at field level were analysed by Chi-square test.

Results

The LDL sample extracted from egg yolk was composed of about 33.83 g dry matter per 100 g fresh LDL sample. The yield of LDL extract obtained was about 67.07 % of whole egg yolk.

The conception rates recorded in group I (egg yolk), II (6% LDL), III (8% LDL) and IV (10% LDL) using ultrasonography on day 30 were 40 percent, 40 percent, 53.33 percent and 33.33 percent respectively. The overall conception rate obtained was 41.67 percent. Statistical analysis using Chi-square test revealed non-significant difference in the conception rates between the four groups (χ^2 value = 1.225; P = 0.7471) (Table1 and Fig. 1). However, it was found that 8% LDL non-significantly improved the conception rate.

Table 1: Conception rate obtained with different groups of semen extenders

Group	No. of AI done	No. of animals conceived	Conception rate (%)
Group I (EY)	15	6	40
Group II (6% LDL)	15	6	40
Group III (8% LDL)	15	8	53.33
Group IV (10% LDL)	15	5	33.33
Total	60	25	41.67
Chi-square, df	1.225, 3		
P value	0.7471		

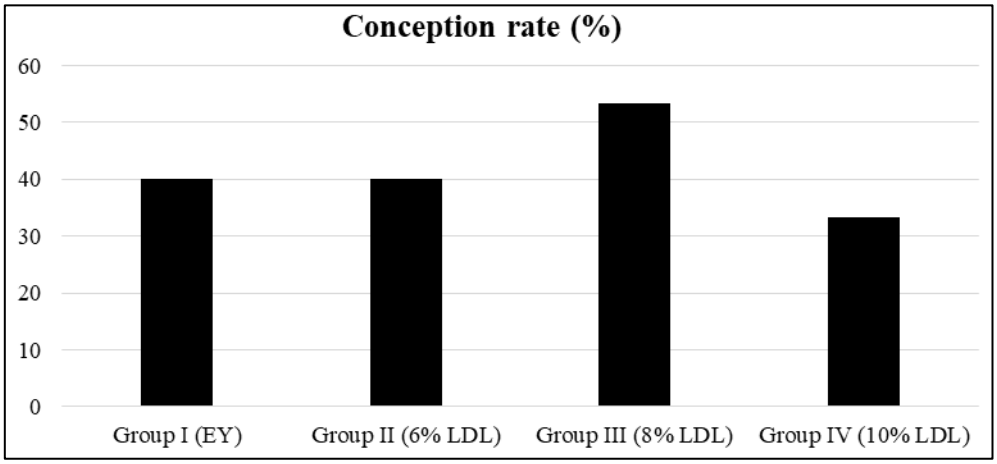

Fig 1: Conception rate obtained with different groups of semen extenders

Discussion

In few studies on effect of LDL on cryopreserved buck semen, it has been found that 8% LDL improved the quality of frozen semen compared to egg yolk and other concentrations of LDL. The review of the literature revealed that there were no previous reports on the effect of replacing egg yolk with LDL in buck semen extender on conception rates obtained by artificial insemination at field level.

However, substitution of egg yolk with 8% LDL significantly (P<0.05) improved the post thaw sperm progressive velocity and acrosomal integrity in frozen-thawed caprine semen, whereas both egg yolk and LDL had similar effect on cell membrane integrity of spermatozoa (Al Ahmad *et al*., 2008) [2]. Another study concluded that addition of 8% LDL to the buck semen extender provided better cryoprotection with significantly (P<0.05) higher post thaw sperm motility, plasma membrane integrity, sperm viability, acrosomal integrity and sperm abnormalities than the extender with whole egg yolk, 4 percent, 6 percent and 10 percent LDL (El-Bawab *et al*., 2015) [8] and 4 percent LDL showed least values

for all the above parameters. It was also found that there was no significant difference between the parameters of semen cryopreserved with egg yolk and 10% LDL.

Few comparative effect of LDL and egg yolk at field level in other species have showed that better fertility was achieved with different concentrations of LDL in different species. LDL at 10 percent concentration produced higher (P<0.01) fertility rate after inseminations with Nili Ravi buffalo bull semen than whole egg yolk and also post thaw seminal parameters were significantly higher in extender containing 10 per cent LDL (Akhter *et al*., 2011) [1]. While, higher (P<0.05) fertility rates were recorded in extender containing 12% LDL (72.7%) than in egg yolk extender (50%) and it was concluded that 12 percent LDL in extender improved the freezability and fertility of buffalo bull spermatozoa (El-Sharawy *et al*., 2012) [9]. In another study, Amirat *et al*. (2004) obtained higher cleavage rate after *in vitro* fertilization using bull semen frozen with 8% LDL than with commercial extender containing egg yolk.

Evidence indicates that the LDL extracted from egg yolk provides protection either by preventing the loss of membrane phospholipids (Parks and Graham, 1992) [12] or by associating with sperm membrane (Bellin *et al*., 1998) [6] thus, increasing the sperm tolerance to the cold shock. Other researchers have proposed that phospholipids from LDL replace some of the sperm membrane's phospholipids, thereby decreasing their phase transition temperature and thus reducing the formation of ice crystals (Moussa *et al*., 2002) [11]. The phospholipids released in the extender, following the disruption of LDL during the freeze-thawing process, could form a gel-like protective film on the spermatozoa, which protects the lipid–protein complex of cell membranes and thereby safeguards the spermatozoa (Akhter *et al*., 2011) [1].

Recent studies suggested that the LDL interacts specifically with the major BSP proteins of seminal plasma. Manjunath *et al*. (2002) [10] and Bergeron *et al*. (2004) [7] hypothesized that the main mechanism whereby LDL protects spermatozoa was through the sequestration of BSP proteins in seminal plasma, considering that the major BSP proteins bind to the sperm surface at ejaculation, triggering cholesterol and phospholipids efflux from the sperm membrane. GSP proteins (homologous to BSP proteins) present in goat seminal plasma also showed the same affinity and the property of binding to the low density fraction of egg yolk similar to BSP proteins (Villemure *et al*., 2003) [15]. These major proteins in goat seminal plasma, though essential for sperm capacitation are detrimental for sperm preservation as they induce a continual phospholipids and cholesterol efflux from sperm membranes.

Binding of LDL to these proteins is rapid and stable even after freeze thawing process and it protects sperm from deleterious effects of BSP proteins and their homologs in other species (Anand *et al*., 2015) [4]. Ultimately, LDL in semen extender improved the physico-morphological attributes of spermatozoa, reduced leakage of intracellular enzymes and oxidative stress on spermatozoa and protected the structural and functional integrity of spermatozoa efficiently as compared to whole egg yolk (Perumal *et al*., 2017) [13].

In the present study, it is shown that 8 percent LDL used to substitute whole egg yolk in semen extender for Osmanabadi buck semen insignificantly improved fertility compared to whole egg yolk and other LDL concentrations. Thus, further studies with larger sample size at field level are recommended for more significant results.

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